REMARKS

Claims 1 and 26-29 are pending in the application. Claim 1 has been amended to further define the scope of the invention. Support for this amendment is found in the specification at page 1, lines 13-27. The Examiner rejected claims 1 and 26-29 under 35 U.S.C. § 102(e), as allegedly anticipated by U.S. Patent No. 5,266,683 (Oppermann). Applicants traverse.

The pending application claims priority to Serial No. 07/525,357, filed on May 16, 1990. Oppermann was not filed until October 18, 1990. Thus, Oppermann is not prior art to the claimed invention.

The Examiner has declined to accord the benefit of this priority applicatoin because the U.S.P.T.O.'s copy of the application file is missing. At the invitation of the Examiner, Applicants have submitted a copy of their records for application Serial No. 07/525,357 so that the U.S.P.T.O. can reconstruct it's file. For convenience, a copy of the '357 application as filed is attached to this response.

The Examiner has acknowledged that the pending claims are entitled to the filing date of international application PCT/US91/03388 and U.S. application Serial No. 07/800,364, filed May 15, 1991 and November 26, 1991, respectively. Both of these applications were copending with, and claim priority to, application Serial No. 07/525,357.

Moreover, the pending claims are fully supported by application Serial No. 07/525,357. Applicants direct the Examiner's attention to, for example, page 1, lines 10-24; page 4, lines 14-19; page 14, lines 4-13; and page 4, lines 22-24. In view of this support and the continuity of disclosure from application Serial No. 07/525,357 to

PCT/US91/03388 and U.S. Application Serial No. 07/800,364 to the pending application, the pending claims should be accorded an effective filing date of May 16, 1990.

Accordingly, Applicants request that the Examiner withdraw the rejection of claims 1 and 26-29 under 35 U.S.C. § 102(e).

In view of the foregoing remarks, Applicant respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: May 24, 2004

By: 
Elizabeth E. McNamee
Reg. No. 54,696

Attachments: Copy of 07/525,357 application as filed.

BONE AND CARTILAGE INDUCTIVE PROTEINS

The present invention relates to a family of purified proteins which may exhibit the ability to induce cartilage and/or bone formation and processes for obtaining them. These proteins may be used to induce bone and/or cartilage formation and in wound healing and tissue repair.

The invention provides a novel family of proteins termed BMP-8 proteins (wherein BMP is Bone Morphogenic Protein). These proteins are capable of stimulating, promoting or otherwise inducing cartilage and/or bone formation. BMP-8 proteins of the invention are characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

(2). Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys

In sequence (2). (Ser) indicates that the residue is not yet absolutely identified, but may be serine.

The BMP-8 proteins of the invention may be further characterized by an apparent molecular weight of 28,000-

38,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Under reducing conditions in SDS-PAGE the protein reveals a region of approximately 14,000-20,000 daltons.

It is contemplated that the proteins of the invention are capable of inducing cartilage and/or bone formation.

The amino acid sequences set forth above are derived from a bovine bone preparation as further described herein. Based on knowledge of other "BMP" proteins it is expected that the human sequence is the same or homologous thereto.

The invention further includes methods for obtaining the DNA sequences encoding the BMP-8 proteins of the invention. This method entails utilizing the above amino acid sequences or portions thereof to design probes to screen libraries for the human gene or fragments thereof using standard techniques.

The proteins of the invention may be produced by culturing a cell transformed with a DNA sequence encoding the BMP-8 protein and recovering and purifying from the culture medium a protein characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr--Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

(2). Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-

Asn-Val-Ile-Leu-Arg

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys

The expressed protein is isolated, recovered and purified from the culture medium. The purified expressed protein is substantially free from other proteinaceous materials with which it is co-produced, as well as from other contaminants. The recovered purified protein is contemplated to exhibit cartilage and/or bone formation activity.

The proteins of the invention may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below. It is further contemplated that the proteins of the invention demonstrate activity in this rat bone formation assay at a concentration of .5μ - 100μg/gram of bone formed. It is further contemplated that these proteins demonstrate activity in this assay at a concentration of 1μg - 50μg/gram bone. More particularly, it is contemplated these proteins may be characterized by the ability of 1μg of the protein to score at least +2 in the rat bone formation assay.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of a protein of the invention in a pharmaceutically acceptable vehicle or carrier. The compositions of the invention may be

used to induce bone and/ or cartilage formation. These compositions may also be used for wound healing and tissue repair. Further compositions of the invention may include in addition to a protein of the present invention at least one other therapeutically useful agent such as the proteins designated BMP-1, BMP-2 (also sometimes referred to as BMP-2A or BMP-2 Class II), BMP-3, BMP-4 disclosed in PCT published applications WO 88/00205 and WO 89/10409, BMP-5, BMP-6, and BMP-7 disclosed in USSN's 437,409, 490,033, and 438,919 filed November 15, 1989, November 15, 1989 and November 17, 1989 respectively. Other therapeutically useful agents include growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factors (TGF-α and TGF-β). The compositions of the invention may also include an appropriate matrix, for instance, for supporting the composition and/or providing a surface for bone and/or cartilage growth. The matrix may provide slow release of the BMP protein and or the appropriate environment for presentation of the BMP protein.

The compositions may be employed in methods for treating a number of bone and/or cartilage defects, and periodontal disease. They may also be employed in methods for treating various types of wounds and in tissue repair. These methods, according to the invention, entail administering to a patient needing such bone and/or cartilage formation, wound healing or tissue repair, a therapeutically effective amount of a

protein of the invention. These methods may also entail the administration of a protein of the invention in conjunction with at least one of the "BMP" proteins disclosed in the co-owned applications described above. In addition, these methods may also include the administration of a protein of the invention with other growth factors including EGF, FGF, TGF-α, and TGF-β.

Still a further aspect of the invention are DNA sequences coding for expression of a BMP-8 protein of the invention. Such sequences include a sequence of nucleotides encoding at least one of the same or substantially the same peptide sequences reported above or fragments thereof.

A further aspect of the invention provides vectors containing a DNA sequence encoding BMP-8 proteins of the invention as described above in operative association with an expression control sequence therefor. Host cells transformed with such vectors for use in producing BMP-8 proteins are also provided by the present invention. The host cells containing DNA sequences encoding BMP-8 may be employed in a novel process for producing a protein of the invention. The transformed host cells are cultured in a suitable culture medium and a protein of the invention is isolated and purified from the cells, cell lysate, or conditioned medium by conventional techniques. This process may employ a number of known cells, both prokaryotic and eukaryotic, as host cells for expression of the polypeptide.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

Brief Description of the Drawing

FIG. 1 illustrates an SDS-PAGE analysis of an oseoinductive

fraction (28,000-38,000 daltons non-reduced) following reduction with dithiolthreitol.

Detailed Description of the Invention

A purified BMP-8 cartilage/bone protein of the present invention is characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr--Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

(2). Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys

Purified BMP-8 proteins are substantially free from proteinaceous materials with which they are co-produced as well as from other contaminants. These proteins may be further characterized by the ability to induce cartilage and/or bone formation. It is contemplated that this activity may be demonstrated by activity in the rat bone formation assay as described in Example III. It is further contemplated that these proteins demonstrate activity in the assay at a concentration of 10μg - 500μg/gram of bone formed. The proteins may be further characterized by the ability of 1μg to score at least +2 in this assay using either the original or modified scoring method.

The proteins of the invention are further characterized by an apparent molecular weight of 28,000 - 38,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Under reducing conditions in

SDS-PAGE the protein reveals a region of approximately 14,000-20,000 daltons.

In a further aspect, the invention provides a method for obtaining the DNA sequences encoding BMP-8 bone/cartilage proteins of the invention. The method for obtaining the DNA sequences entails utilizing the amino acid sequences describing above to design probes to screen using standard techniques. The bovine sequence or the human gene thus identified may also be used as a probe to identify a human cell line or tissue which synthesizes the analogous cartilage/bone protein. A cDNA library is synthesized and screened with probes derived from the human or bovine coding sequences. The human sequence thus identified is transformed into a host cell, the host cell is cultured and the protein recovered, isolated and purified from the culture medium. The purified protein is predicted to exhibit cartilage and/or bone formation activity in the rat bone formation assay of Example III.

The proteins provided herein also include factors encoded by the above described sequences but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. Similarly, synthetic polypeptides which wholly or partially duplicate continuous sequences of the amino acid residues of the proteins of the BMP-8 proteins are encompassed by the invention. These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with other cartilage/bone proteins of the invention may possess bone and/or cartilage growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring proteins in therapeutic processes.

Other specific mutations of the sequences of the proteins of the invention described herein involve

modifications of the glycosylation site. These modifications may involve O-linked or N-linked glycosylation sites. For instance, the absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at the asparagine-linked glycosylation recognition sites present in the sequences of the proteins of the invention. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Expression of such altered nucleotide sequences procedures variants which are not glycosylated at that site.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for the proteins of the invention. Further included are those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences isolated in accordance with the procedure described above and demonstrate cartilage and/or bone formation activity in the rat bone formation assay. An example of one such stringent hybridization condition is hybridization at 4 x SSC at 65°C, followed by a washing in 0.1 x SCC at 65°C for an hour. Alternatively, an exemplary stringent hybridization condition is in 50% formamide, 4 x SCC at 42°C.

Similarly, DNA sequences isolated as described above which encode BMP-8 proteins, but which differ in codon

sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the proteins of the invention described herein. Variations in the DNA sequences which are caused by point mutations or by induced modifications (including insertion, deletion, and substitution) to enhance the activity, half-life or production of the polypeptides encoded thereby are also encompassed in the invention.

Another aspect of the present invention provides a novel method for producing the proteins of the invention. This method involves culturing a suitable cell line, which has been transformed with a DNA sequence coding for expression of a protein of the invention, under the control of known regulatory sequences. Regulatory sequences include promoter fragments, terminator fragments and other suitable sequences which direct the expression of the BMP-8 protein in an appropriate host cell. A purified BMP-8 protein of the present invention is recovered, isolated and purified from the culture medium. The purified protein is characterized by comprising at least one of the same or substantially the same amino acid sequences comprising

(1). Arg-His-Glu-Leu-Tyr--Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

(2). Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and

purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. The mammalian cell CV-1 may also be suitable. Further exemplary mammalian host cells include particulary primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to , HeLa, mouse L-929 cells, 3T3 lines derived form Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Bacterial cells may also be suitable hosts. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of the proteins of the invention. Preferably the vectors contain the full novel BMP-8 DNA sequences described above which code for the novel cartilage/bone proteins of the invention. Additionally, the vectors also contain appropriate expression control sequences

permitting expression of the protein sequences. Alternatively, vectors incorporating truncated or otherwise modified sequences as described above are also embodiments of the present invention and useful in the production of the proteins of the invention. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Useful regulatory sequences for such vectors are known to those skilled in the art and may be selected depending upon the selected host cells. Such selection is routine and does not form part of the present invention. The components of the vectors, e.g. replicons, selection genes, enhancers, promoters, and the like, may be obtained from natural sources or synthesized by known procedures. See, Kaufman et al, J. Mol. Biol., 159:511-521 (1982); and Kaufman, Proc. Natl. Acad. Sci., USA, 82:689-693 (1985). Host cells transformed with such vectors and progeny thereof for use in producing cartilage/bone proteins are also provided by the invention.

A protein of the present invention, which induces cartilage and/or bone formation in circumstances where bone and/or cartilage is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. A protein of the invention may be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract

bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See, e.g. PCT Publication WO84/01106 for discussion of wound healing and related tissue repair).

A further aspect of the invention includes therapeutic methods and composition for repairing fractures and other conditions related to bone and/or cartilage defects or periodontal diseases. In addition, the invention comprises therapeutic methods and compositions for wound healing and tissue repair. Such compositions comprise a therapeutically effective amount of at least one of the proteins of the invention in admixture with a pharmaceutically acceptable vehicle, carrier or matrix. It is expected that the proteins of the invention may act in concert with or perhaps synergistically with one another or with other related proteins and growth factors. Therapeutic methods and compositions of the invention therefore comprise one or more of the proteins of the present invention. Further therapeutic methods and compositions of the invention therefore comprise a therapeutic amount of at least one protein of the invention with a therapeutic amount of at least one of the other "BMP" proteins disclosed in co-owned and co-pending U.S. applications described above. Such methods and compositions of the invention may comprise proteins of the invention or portions thereof in combination with the above-mentioned "BMP" proteins or portions thereof. Such combination may comprise individual molecules from each of the proteins or heteromolecules formed by portions of the respective proteins. For example, a method and composition

of the invention may comprise a protein of the invention or a portion thereof linked with a portion of a "BMP" protein to form a heteromolecule.

Further therapeutic methods and compositions of the invention comprise the proteins of the invention or portions thereof in combination with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF). Portions of these agents may also be used in compositions of the invention.

The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art. The therapeutic compositions are also presently valuable for veterinary applications due to the apparent lack of species specificity in cartilage and bone growth factor proteins. Domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the proteins of the present invention.

The therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of cartilage and/or bone or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the cartilage/bone proteins of the invention to the site of bone and/or cartilage damage, providing a structure for the developing

bone and cartilage and optimally capable of being reabsorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions of the invention will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of the proteins of the invention. Factors which may modify the action of the proteins of the invention include the amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the type or types of bone and/or cartilage proteins present in the composition. The addition of other known growth factors,

such as EGF, PDGF, TGF-α, TGF-β, and IGF-I to the final composition, may also effect the dosage.

Progress can be monitored by periodic assessment of cartilage and/or bone growth and/or repair. The progress can be monitored, for example, using x-rays, histomorphometric determinations and tetracycline labeling .

The following examples illustrate practice of the present invention in recovering and characterizing bovine cartilage and/or bone proteins of the invention and employing these proteins to recover the corresponding human protein or proteins and in expressing the proteins via recombinant techniques.

Example I

Isolation of Bovine Cartilage/Bone Inductive Protein

Ground bovine bone powder (20-120 mesh, Colla-Tec) is prepared according to the procedures of M. R. Urist et al., Proc. Natl Acad. Sci USA, 70:3511 (1973) with elimination of some extraction steps as identified below. Ten kgs of the ground powder is demineralized in successive changes of 0.6N HCl at 4°C over a 48 hour period with vigorous stirring. The resulting suspension is extracted for 4 hours in 26 liters of 0.5M EDTA. The residue is washed two times with distilled water before its resuspension in 10 liters of 4M guanidine hydrochloride [GuCl], 1mM N-ethylmaleimide, 1mM iodoacetic acid, 1mM phenylmethylsulfonyl fluoride as described in Clin. Orthop. Rel. Res., 171: 213 (1982). After 16 to 20 hours the supernatant is removed and replaced with another 6 liters of GuCl buffer. The residue is extracted for another 8 hours. The final extraction with 6 liters of GuCl is carried out for 16 hours.

The crude GuCl extracts are combined, filtered through a Pellicon apparatus with a 0.45mM Durapore tangential flow filter packet, concentrated approximately 50 times on a Amicon RA2000 apparatus with a 10,000 molecular weight

cut-off membrane, and then dialyzed in 20mM Tris, 0.05M NaCl, 6M urea (pH7.1), the starting buffer for the first column. After extensive dialysis the protein is loaded on a 2 liter DEAE cellulose column and the unbound fractions are collected.

The unbound fractions are concentrated and dialyzed against 50mM NaAc, 50mM NaCl (pH 4.6) in 6M urea. The unbound fractions are applied to a carboxymethyl cellulose column. Protein not bound to the column is removed by extensive washing with starting buffer, and the material containing protein having bone and/or cartilage formation activity as measured by the Rosen-modified Sampath - Reddi assay (described in Example III below) desorbed from the column by 50mM NaAc, 0.25mM NaCl, 6M urea (pH 4.6). The protein from this step elution is concentrated 20- to 40-fold, then dialyzed extensively against 80mM KPO_4, 6M urea (pH6.0). The sample is applied to an hydroxylapatite column (IBF) equilibrated in 80mM KPO_4, 6M urea (pH6.0) and all unbound protein is removed by washing the column with the same buffer. Protein having bone and/or cartilage formation activity is eluted with 100mM KPO_4 (pH7.4) and 6M urea.

The protein is diluted 5 fold with a 0.1875 M NaCl, 6 M urea solution to a final concentration of 20 mM KPO_4, 150 mM NaCl, 6 M urea. This material is applied to a heparin - Sepharose column equilibrated in 20mM KPO_4, 150mM NaCl, 6M urea (pH7.4). After extensive washing of the column with starting buffer, a protein with bone and/or cartilage inductive activity is eluted by 20mM KPO_4, 700mM NaCl, 6M urea (pH7.4). This fraction is concentrated 10 - 20 fold, dialyzed against 50mM NaAc, 6M urea (pH4.6), and applied to a Pharmacia MonoS HR column. The column is developed with a gradient to 1.0M NaCl, 50mM NaAc, 6M urea (pH4.6). All fractions with absorbance at 280 mM are pooled. This Mono S step is now believed to be dispensable and will be eliminated in the future. The material is applied to a 4.7 x 30 cm

Waters PrepPak 500 C4 cartridge in 0.1% TFA and the column developed wit a gradient to 95% acetonitrile, 0.1% TFA in 100 minutes at 45ml per minute. Fractions were assayed for cartilage and/or bone formation activity.

Aliquots of the appropriate fractions are iodinated by one of the following methods: P. J. McConahey et al, Int. Arch. Allergy, 29:185-189 (1966); A. E. Bolton et al, Biochem J., 133:529 (1973); and D. F. Bowen-Pope, J. Biol. Chem., 237:5161 (1982). The iodinated proteins present in these fractions are analyzed by SDS gel electrophoresis.

EXAMPLE II

Characterization of Bovine Cartilage/Bone Inductive Factor

A. Molecular Weight

Approximately 2.5mg protein from Example I from active BMP containing fractions in 0.1% TFA and approximately 45% acetonitrile, is dried with a savant Speed Vac concentrator and solubilized with Laemmli sample buffer, loaded onto a 12.5% polyacrylamide gel and subjected to SDS-PAGE [Laemmli, U.K. Nature, 227:680-685 (1970)] without reducing the sample with dithiothreitol. The molecular weight is determined relative to iodinated Bio-Rad molecular weight standards. Following autoradiography of the unfixed gel the approximate 28,000-38,000 dalton band is excised and the protein electrophoretically eluted from the gel (Hunkapillar et al Meth. Enzymol. 91:227-236 (1983)]. Based on similar purified bone fractions as described in the co-pending "BMP" applications described above wherein bone and/or cartilage activity is found in the approximately 28,000-38,000 region, it is inferred that this band comprises bone and/or cartilage inductive fractions.

B. Subunit Characterization

The subunit composition of the isolated bovine bone

protein is also determined. The eluted protein described above is fully reduced and alkylated in 2% SDS using iodoacetate and standard procedures. The fully reduced and alkylated sample is then further submitted to SDS-PAGE on a 12.5% gel and the resulting approximate 14,000-20,000 dalton region having a doublet/triplet appearance located by autoradiography of the unfixed gel. A silver stain [Merril et al, Science, 211 : 1437 (1981)] version of the sample is shown in FIG. 1 along with molecular weight markers. The 14,000-20,000 dalton region is indicated by the bracket. Thus the approximate 28,000-30,000 dalton protein yields a broad region of 14,000-20,000.

EXAMPLE III

Rosen Modified Sampath-Reddi Assay

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. U.S.A., 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of the proteins of the invention. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved in 0.1 % TFA, and the resulting solution added to 20mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male Long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi

et al., Proc. Natl Acad Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. Glycolmethacrylate sections (1μm) are stained with Von Kossa and acid fuschin or toluidine blue to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and newly formed bone and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2 and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone.

It is contemplated that the dose response nature of the cartilage and/or bone inductive protein containing samples of the matrix samples will demonstrate that the amount of bone and/or cartilage formed increases with the amount of cartilage/bone inductive protein in the sample. It is contemplated that the control samples will not result in any bone and/or cartilage formation.

As with other cartilage and/or bone inductive proteins such as the above-mentioned "BMP" proteins, the bone and/or cartilage formed is expected to be physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing followed by autoradiography. The activity is correlated with the protein bands and pI. To estimate the purity of the protein in a particular fraction an extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and the protein is run on SDS PAGE followed by silver staining or radioiodination and autoradiography.

EXAMPLE IV

Bovine Protein Composition

The gel slice of the approximate 14,000-20,000 dalton region described in Example IIB is excised and the protein electrophoretically eluted from the gel (Hunkapillar, et al., Supra.). This isolated protein sample is then depleted of SDS [Simpson, et al., Eur. J. Biochem. 165:21-29 (1987)] by being bound to a 30 x 2.1 mm Brownlee RP-18 after dilution with 5 volumes of 90% n-propanol. Protein is recovered by eluting with a step of 40% n-proponal, 0.1% TFA. The fractions containing the eluted protein peak are pooled and brought to near dryness in a savant Speed Vac concentrator. The protein is then re-solubilized with 0.1 M ammonium bicarbonate and digested with 1 μg of TPCK - treated trypsin (Worthington) for 16 hours at 37°C. A second 1 μg dose of trypsin was added and digestion continued for another 4 hours. The resultant digest is then subjected to RPHPLC using a C4 Vydac RPHPLC column and 0.1% TFA-water, 0.1% TFA water-acetonitrile gradient. The resultant peptide peaks were monitored by UV absorbance at 214 and 280 nm and subjected to direct amino terminal amino acid sequence analysis using an Applied Biosystems gas phase sequenator (Model 470A). Three tryptic fragments are isolated by standard procedures having the following amino acid sequence as represented by the amino acid standard three-letter symbols and where the amino acid in parentheses indicates uncertainty in the sequence:

(1). Arg-His-Glu-Leu-Tyr--Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

(2). Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg

(3). Ala-Cys-Cys-Ala-Pro-Thr-Lys

The two amino acid sequences identified above share

homology with other BMP proteins BMP-2, BMP-3, BMP-4, BMP-4 disclosed in PCT published applications WO 88/00205 and WO 89/10409, BMP-5, BMP-6, and BMP-7 disclosed in USSN's 437,409, 490,033, and 438,919 filed November 15, 1989, November 15, 1989 and November 17, 1989 respectively. Specifically, the above amino acid sequence

(1). Arg-His-Glu-Leu-Tyr--Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

shares homology with BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7 which contain the following human homologous sequence:

BMP-2: Arg-His-Pro-Leu-Tyr-Val-Asp-Phe-Ser-Asp-Val-Gly-Trp-Asn-Asp-Trp-Ile-Val-Ala-Pro-Pro-Gly-Tyr

BMP-3: Arg-Arg-Tyr-Leu-Lys-Val-Asp-Phe-Ala-Asp-Ile-Gly-Trp-Ser-Glu-Trp-Ile-Ile-Ser-Pro-Lys-Ser-Phe

BMP-4: Arg-His-Ser-Leu-Tyr-Val-Asp-Phe-Ser-Asp-Val-Gly-Trp-Asn-Asp-Trp-Ile-Val-Ala-Pro-Pro-Gly-Tyr

BMP-5: Lys-His-Glu-Leu-Tyr-Val-Ser-Phe-Arg-Asp-Leu-Gly-Trp-Gln-Asp-Trp-Ile-Ile-Ala-Pro-Glu-Gly-Tyr

BMP-6: Lys-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Gln-Asp-Trp-Ile-Ile-Ala-Pro-Lys-Glu-Tyr

BMP-7: Lys-His-Glu-Leu-Tyr-Val-Ser-Phe-Arg-Asp-Leu-Gly-Trp-Gln-Asp-Trp-Ile-Ile-Ala-Pro-Glu-Gly-Try

The second amino acid sequence

(2). Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg.

shares homology with the following human sequences of these BMP molecules:

BMP-2: Leu-Ser-Ala-Ile-Ser-Met-Leu-Tyr-Leu-Asp-Glu-Asn-Glu-Lys-Val-Val-Leu-Lys

BMP-3: Met-Ser-Ser-Leu-Ser-Ile-Leu-Phe-Phe-Asp-Glu-Asn-Lys-Asn-Val-Val-Leu-Lys

BMP-4: Leu-Ser-Ala-Ile-Ser-Met-Leu-Tyr-Leu-Asp-Glu-Tyr-Asp-Lys-Val-Val-Leu-Lys

BMP-5: Leu-Asn-Ala-Ile-Ser-Val-Leu-Tyr-Phe-Asp-Asp-Ser-Ser-Glu-Val-Ile-Leu-Lys

BMP-6: Leu-Asn-Ala-Ile-Ser-Val-Leu-Tyr-Phe-Asp-Asp-Asn-Ser-Asn-Val-Ile-Leu-Lys

BMP-7: Leu-Asn-Ala-Ile-Ser-Val-Leu-Tyr-Phe-Asp-Asp-Ser-Ser-Asn-Val-Ile-Leu-Lys

The third amino acid sequence (3). Ala-Cys-Cys-Ala-Pro-Thr-Lys shares homology with the following human sequences of these BMP molecules:

BMP-2: Ala-Cys-Cys-Val-Pro-Thr-Glu
BMP-3: Pro-Cys-Cys-Val-Pro-Glu-Lys
BMP-4: Ala-Cys-Cys-Val-Pro-Thr-Glu
BMP-5: Pro-Cys-Cys-Ala-Pro-Thr-Lys
BMP-6: Pro-Cys-Cys-Ala-Pro-Thr-Lys
BMP-7: Pro-Cys-Cys-Ala-Pro-Thr-Gln

It is contemplated that the BMP-8 proteins of the invention will be structurally similar to these BMP proteins BMP-2 through BMP-7.

EXAMPLE V

Isolation of DNA

Isolation of DNA sequences encoding BMP-8 proteins may be isolated using various techniques known to those skilled in the art. For instance, oligonucleotide probes may be designed on the basis of the amino acid sequence of the above-identified tryptic fragments and synthesized on an automatic DNA synthesizer. The probes may consist of pools of oligonucleotides or unique oligonucleotides designed from the tryptic sequences according to the method of R. Lathe, J. Mol. Biol. 183(1):1-12 (1985).

Because the genetic code is degenerate (more than one codon can code for the same amino acid), a mixture of oligonucleotides is synthesized that contains all possible nucleotide sequences encoding the amino acid sequence of the tryptic fragment or portion thereof.

It may be possible in some cases to reduce the number of oligonucleotides in the probe mixture based on codon usage because some codons are rarely used in eukaryotic genes, and because of the relative infrequency of the dinucleotide CpG in eukaryotic coding sequences [see J.J. Toole et al, Nature 312:342-347 (1984)]. The regions of the amino acid sequence used for probe design are chosen by avoiding highly degenerate codons where possible. The oligonucleotides are synthesized on an automated DNA synthesizer and the probes are then radioactively labelled and employed to screen a selected library.

The probes may be employed in various ways for obtaining DNA sequences encoding BMP-8 proteins of the invention using techniques known to those skilled in the art. For instance, probes designed on the above identified amino acid sequences may be used for screening bovine libraries for identifying the bovine DNA sequences. The bovine DNA sequences may then in turn be utilized for screening human libraries.

Bovine cDNA may be synthesized from polyadenylated RNA from a bovine cells. Such RNA may be isolated for instance, by oligo(dT) cellulose chromatography from total RNA isolated

from fetal bovine bone cells by the method of Gehron-Robey et al in Current Advances in Skeletogenesis, Elsevier Science Publishers (1985). A cDNA library is made in lambda gt10 (Toole et al supra) or other suitable vectors using established techniques and plated. Recombinants are screened on nitrocellulose filters with the probes synthesized as described above. Positives are plaque purified, a phage plate stock made, and bacteriophage DNA isolated. This DNA is digested and subcloned into an appropriate vector such as M13 and pSP65 and the DNA sequence and derived amino acid sequence are determined.

If the above procedure results in a partial sequence the full sequence can be obtained using probes designed based on the partial sequence. These probes are used for further screening to obtain the full sequence.

EXAMPLE V

Human Cartilage/Bone Proteins

Bovine and human bone growth factor genes are presumed to be significantly homologous, therefore the bovine coding sequence or a portion thereof is used as a probe to screen a human genomic library or as a probe to identify a human cell line or tissue which synthesizes the analogous human cartilage and/or bone protein. A human genomic library (Toole et al., supra) may be screened with such a probe, and presumptive positives isolated and DNA sequence obtained. Evidence that this recombinant encodes a portion of the human bone inductive factor molecule relies on the bovine/human protein and gene structure homologies.

Once a recombinant bacteriophage containing DNA encoding a portion of the human cartilage and/or bone inductive factor molecule is obtained, the human coding sequence can be used as a probe to identify a human cell line or tissue which synthesizes bone inductive factor.

Alternatively, the bovine coding sequence can be used as a probe to identify such human cell line or tissue. Briefly described, RNA is extracted from a selected cell or tissue source and either electrophoresed on a formaldehyde agarose gel and transferred to nitrocellulose, or reacted with formaldehyde and spotted on nitrocellulose directly. The nitrocellulose is then hybridized to a probe derived from a coding sequence of the bovine or human cartilage and/or bone inductive protein. mRNA is selected by oligo (dT) cellulose chromatography and cDNA is synthesized and cloned in lambda gt10 by established techniques (Toole et al., supra).

Additional methods known to those skilled in the art may be used to isolate the human and other species' cartilage/bone proteins of the invention.

EXAMPLE VI

Expression of the Cartilage/Bone Proteins

In order to produce bovine, human or other mammalian proteins of the invention, the DNA encoding it, isolated as described above, is transferred into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts by conventional genetic engineering techniques. Methods of transfection include electroporation, $CaPO_4$ precipitation, protoplast fusion, microinjection and lipofection. Once the host cells are transformed, stable transformants are then screened for expression of the product by standard immunological, biological or enzymatic assays. The presence of this DNA and mRNA encoding the BMP-8 polypeptides may be detected by standard procedures such as Southern and Northern blotting, high expressing cell lines are cloned or recloned at the appropriate level of selectivity to obtain a more homologous population of cells.

Selected transformed host cells are cultured and the

BMP-8 proteins of the invention expressed thereby are recovered, isolated and purified. Characterization of the expressed proteins is carried out using standard techniques. For example characterization may include pulse labeling with [35^S] methionine or cysteine and analysis by polyacrylamide electrophoresis. The recombinantly expressed BMP-8 proteins are free of proteinaceous materials with which they are coproduced and with which they ordinarily are associated in nature, as well as from other contaminants, such as materials found in the cellular media.

It is contemplated that the preferred expression system for biologically active recombinant human proteins of the invention will be stably transformed mammalian cells. For transient expression the cell line of choices is expected to be SV40 transformed African green monkey kidney COS-1 in COS-7 which typically produce moderate amounts of the protein encoded within the plasmid for a period of 1-4 days. It is further contemplated that the preferred mammalian cells will be CHO cells.

One skilled in the art can construct mammalian expression vectors by employing the DNA sequences of the invention sequences and known vectors, such as pCD [Okayama et al., Mol. Cell Biol., 2:161-170 (1982)] and pJL3, pJL4 [Gough et al., EMBO J., 4:645-653 (1985)]. The transformation of these vectors into appropriate host cells may result in expression of the proteins of the invention. One skilled in the art could manipulate the sequences of the invention by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by bacterial cells. For example, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences there-from or altering nucleotides therein by other known techniques). The

modified coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl Acad. Sci. USA, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and a protein of the invention expressed thereby. For a strategy for producing extracellular expression of a cartilage and/or bone protein of the invention in bacterial cells., see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [See, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See, e.g., procedures described in published PCT application WO86/00639 and European patent application EPA 123,289].

A method for producing high levels of a protein of the invention from mammalian cells involves the construction of cells containing multiple copies of the heterologous gene encoding proteins of the invention. The heterologous gene may be linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982). This approach can be employed with a number of different cell types. For example, a plasmid containing a DNA sequence for a protein of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A)3 [Kaufman and Sharp, Mol. Cell. Biol., 2:1304 (1982)] may be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate

coprecipitation and transfection, electroperation or protoplast fusion. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., Mol Cell Biol., 5:1750 (1983). Transformants are cloned, and the proteins of the invention are recovered, isolated, and purified from the culture medium. Biologically active protein expression is monitored by the Rosen-modified Sampath - Reddi rat bone formation assay described above in Example III. Protein expression should increase with increasing levels of MTX resistance. Similar procedures can be followed to produce other related proteins.

EXAMPLE VII

Biological Activity of Expressed Cartilage/Bone Proteins

To measure the biological activity of the expressed BMP-8 proteins obtained in Example VI above, the protein is partially purified on a Heparin Sepharose column and further purified using standard purification techniques known to those skilled in the art. Post transfection conditioned medium supernatant collected from the cultures is concentrated approximately 10 fold by ultrafiltration on a YM 10 membrane and then dialyzed against 20mM Tris, 0.15 M NaCl, pH 7.4 (starting buffer). This material is then applied to a Heparin Sepharose column in starting buffer. Unbound proteins are removed by an 8 ml wash of starting buffer, and bound proteins, including proteins of the invention, are desorbed by a 3-4 ml wash of 20 mM Tris, 2.0 M NaCl, pH 7.4.

The proteins bound by the Heparin column are concentrated approximately 10-fold on a Centricon 10 and the salt reduced by diafiltration with 0.1% trifluoroacetic acid. The appropriate amount of this solution is mixed with 20 mg of rat matrix and then assayed for in vivo bone and/or

cartilage formation activity by the Rosen-modified Sampath-Reddi assay. A mock transfection supernatant fractionation is used as a control.

Further purification may be achieved by preparative $NaDodSO_4$/PAGE [Laemmli, Nature 227:680-685 (1970)]. For instance, approximately 300 μg of protein is applied to a 1.5-mm-thick 12.5% gel: recovery is be estimated by adding L-[^{35}S]methionine-labeled BMP protein purified over heparin-Sepharose as described above. Protein may be visualized by copper staining of an adjacent lane [Lee, et al., Anal. Biochem. 166:308-312 (1987)]. Appropriate bands are excised and extracted in 0.1% $NaDodSO_4$/20 mM Tris, pH 8.0. The supernatant may be acidified with 10% CF_3COOH to pH 3 and the proteins are desalted on 5.0 x 0.46 cm Vydac C_4 column (The Separations Group, Hesperia, CA) developed with a gradient of 0.1% CF_3COOH to 90% acetonitrile/0.1% CF_3COOH. The implants containing rat matrix to which specific amounts of human proteins of the invention have been added are removed from rats after seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated and scored as described in Example III.

Levels of activity may also be tested for host cell extracts. Partial purification is accomplished in a similar manner as described above except that 6 M urea is included in all the buffers.

The procedures described above may be employed to isolate other related proteins of interest by utilizing the bovine or human proteins as a probe source. Such other proteins may find similar utility in, inter alia, fracture repair, wound healing and tissue repair.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

What is claimed is:

1. A purified protein comprising at least one of the following sequences:

a) Arg-His-Glu-Leu-Tyr-Val-Ser-Phe-Gln-Asp-Leu-Gly-Trp-Leu-Asp-Trp-Val-Ile-Ala-Pro-Gln-Gly-Tyr

b) Leu-(Ser)-Ala-Thr-Ser-Val-Leu-Tyr-Tyr-Asp-Ser-Ser-Asn-Asn-Val-Ile-Leu-Arg.

c) Ala-Cys-Cys-Ala-Pro-Thr-Lys

2. A purified protein produced by the steps of:

(a) culturing a cell transformed with a vector comprising DNA sequence encoding a protein of Claim 1 in operative association with an expression control sequence therefor; and

(b) recovering, isolating and purifying from said culture medium a protein characterized by the ability to induce cartilage and/or bone formation in the Rosen-modified Sampath-Reddi assay.

3. A protein of claim 1 further characterized by the ability to demonstrate cartilage and/or bone formation.

4. A protein of claim 3 further characterized by the ability of 1μg of said protein to score at least +2 in the Rosen-modified Sampath-Reddi assay.

5. A DNA sequence encoding a protein of claim 1.

6. A DNA sequence encoding a protein of claim 3.

7. A host cell transformed with a DNA of claim 5.

8. A method for producing a purified human BMP-8 protein said method comprising the steps of

(a) culturing a cell transformed with a vector comprising DNA sequence encoding a protein of Claim 1 in operative association with an expression control sequence therefor; and

(b) recovering, isolating and purifying from said culture medium a protein characterized by the ability to induce cartilage and/or bone formation in the Rosen-modified Sampath-Reddi assay.

said protein characterized by at least one of the following sequences or a sequence substantially homologous thereto.

9. A pharmaceutical composition comprising an effective amount of a BMP-8 protein in admixture with a pharmaceutically acceptable vehicle.

10. A pharmaceutical formulation for bone and/or cartilage formation comprising an effective amount of a BMP-8 protein in a pharmaceutically acceptable vehicle.

11. A composition of claim 9 further comprising a matrix for supporting said composition and providing a surface for bone and/or cartilage formation.

12. The composition of claim 11 wherein said matrix comprises a material selected from the group consisting of hydroxyapatite, collagen, polylactic acid and tricalcium phosphate.

13. A method for inducing bone and/or cartilage formation in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 9.

14. A pharmaceutical composition for wound healing and tissue repair said composition comprising an effective amount of a BMP-8 protein in a pharmaceutically acceptable vehicle.

15. A method for treating wounds and/or tissue repair in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 14.

16. A vector comprising a DNA sequence of Claim 5 in operative association with an expression control sequence therefor.

17. A vector comprising a DNA sequence of Claim 4 in operative association with an expression control sequence therefor.

18. A host cell transformed with a vector sequence of Claim 17.

19. A host cell transformed with a vector sequence of Claim 18.

20. A method for producing a BMP-8 protein, said method comprising the steps of

(a) culturing in a suitable culture medium said transformed host cell of claim 18; and

(b) isolating and purifying said bone and/or cartilage inductive protein from said culture medium.

ABSTRACT

Purified cartilage and/or bone inductive proteins and processes for producing them are disclosed. The proteins may be used in the treatment of bone and/or cartilage defects and in wound healing and related tissue repair.